SEXUAL DIMORPHISM IN MALPIGHIAN TUBULES OF *PTERONARCYS CALIFORNICA* NEWPORT (PLECOPTERA)

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ABSTRACT.— The Malpighian tubules in female nymphs and adults of *Pteronarcys californica* Newport are larger and whiter than in males. This difference is detectable in nymphs as small as 300 mg in live weight (final instar nymphs weigh 800 to 1600 mg depending on sex), and is most pronounced in late nymphs and adults. These differences are thought to be related to the increased excretory load imposed by the synthesis of ooplasm in the late female nymph and adult.

The excretory systems of Plecoptera reflect the relatively primitive taxonomic level of this order. The Malpighian tubules are usually numerous and unbranched and have an uncomplicated relationship to the gut, joining it at the level where the midgut empties into the hindgut. Apart from this connection, the tubules are mainly free and unattached, extending throughout the body cavity. This situation is seen with textbook clarity in *Pteronarcys californica.*

Much current interest in the Malpighian tubules centers on their ultrastructure and on their function as a homeostatic organ (Berridge and Oschman 1969; Phillips and Maddrell 1974). They are involved in the elimination of salts and amines, and in water balance. Most studies have been done on terrestrial forms in which water retention and ion transport are achieved in specializations of the tubules and, at times, inclusion of tubules in the wall of the hind gut. The only studies on excretion in Plecoptera are those of Colby (1972), who used *in vivo* measurements to demonstrate the formation of a dilute urine in the aquatic nymphs of *Pteronarcys californica.* There have been no detailed morphological studies of Plecopteran Malpighian tubules.

In a study of the growth of internal organs (Branham and Hathaway 1975), I have observed that there is a distinct sexual dimorphism in the appearance of Malpighian tubules in *P. californica,* which has not to my knowledge been reported earlier.

METHODS

Stoneflies were collected in the Provo River in Utah and transported alive to the laboratory. Several hundred fresh and preserved animals from all size classes were examined. The dimorphisms were generally well preserved in animals fixed in 70 percent ethanol and other killing and fixing fluids. A few animals were used for histological preparations in which 10μ sections were stained with Delafields hematoxylin. Weights of live animals were taken on an analytical balance after thorough blotting to remove excess water.

OBSERVATIONS

Male nymphs in the final instars weigh, on the average, about 800 mg, whereas the corresponding female nymphs are about twice as big, or 1600 mg. The Malpighian tubules in the female nymphs in all size classes above 300 mg are larger and whiter than those in the males. This difference becomes more pronounced as the animals grow larger and is at its maximum in the final instars and adults (compare Figs. 1a & c). The differences are not obvious in animals weighing less than 300 mg.

It is at about 300 mg of body weight that the body-size differences between male and female nymphs becomes discernable; therefore the onset of differences in the Malpighian tubules corresponds with this event. It is not merely size, however, that is involved in the sexual dimorphism in the Malpighian tubules. In females the tubules are boldly conspicuous because of their intense whiteness, whereas the male tubules are at first difficult to find because of their translucent, colorless or pink-tinged appearance. The female tubules, moreover, are of more uniform diameter throughout their length, while the diameter of male tubules is irregular. Females that are smaller and at least a year younger than final instar males still have tubules that

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are larger and whiter (compare Figs. 1a & b).

About 20 percent of the tubules in animals of both sexes are connected anteriorly to the midgut by their distal ends (Fig. 1a). This union is formed from a delicate strand of solid connective tissue, so there is no confluence at this point be-

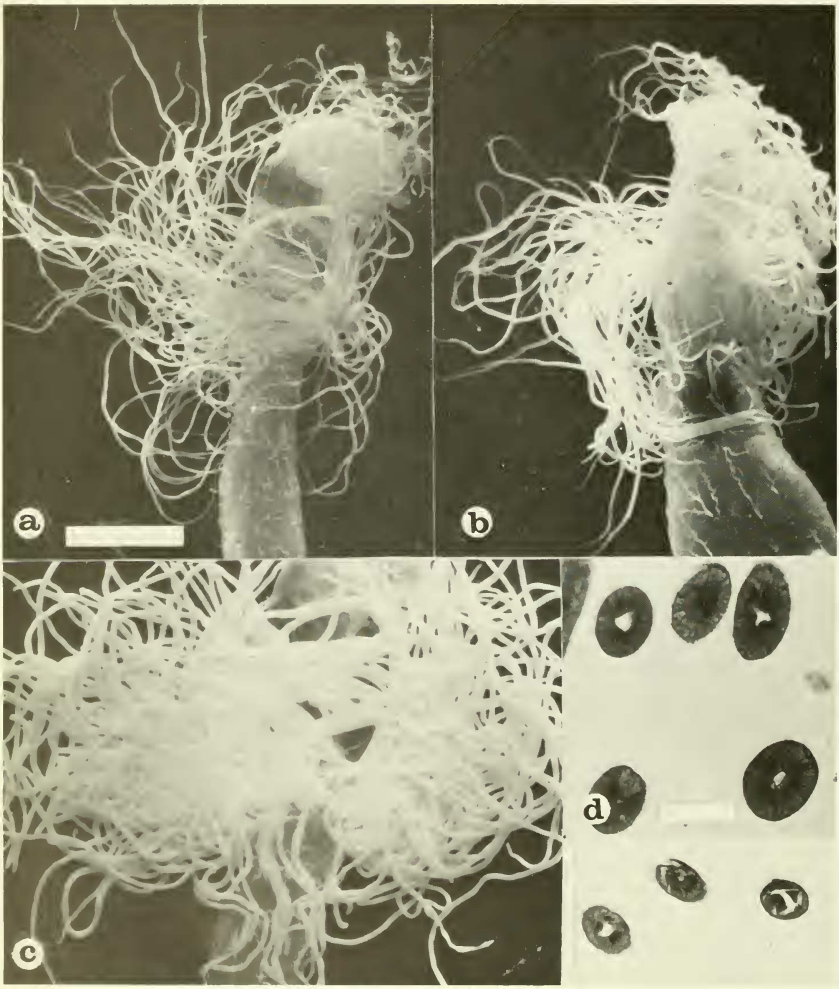

Fig. 1: Malpighian tubules in *Pteronarcys californica*: (a) Male final-year nymph. Live wt. = 840 mg. Isolated gut with anterior end down. Some of the tubules are anchored to the gut by their distal ends. Scale bar equals 2 mm. (b) Female penultimate-year nymph. Live wt. = 620 mg. Isolated gut with anterior end down. Tubules are larger and whiter than in (a). Anchored distal ends of some of the tubules are again visible. Magnification same as in (a). (c) Female final-year nymph. Live wt. = 1560 mg. Isolated gut with anterior end down. Magnification same as in (a) and (b). (d) Female final-year nymph. Cross sections of tubules. Scale bar = 0.1 mm. (e) Male final-year nymph. Cross sections of tubules. Magnification is same as in (d).

tween the lumina of the gut and the tubules. The lumina of these tubules drain into the gut at the same level as all the other tubules, i.e. at the point of junction between the midgut and the hindgut. Between their proximal and distal ends, these tubules are totally free of the gut wall; the only obvious difference from the majority of the Malpighian tubules is that their distal ends are anchored to the midgut at a point about two-thirds of the way from its anterior end, while the distal ends of the other tubules are free.

Discussion

The differences described above must have a functional basis relating to sex. Gonadal development can be seen in both sexes as they begin their final year of nymphal life. At this time males weigh about 500 mg and females weigh 900 to 1000 mg. Gonadal enlargement and sperm production are initiated early in the male; maximum gonadal size is reached midway through the final year. Corresponding events in the female do not occur until the final six or eight weeks of nymphal life. During this period a large part of metabolic effort in females is directed toward the synthesis of ooplasm. There is evidence that oogenesis utilizes substances from the fat body (Branham and Hathaway 1972). This effort continues into the adult stage, in which gamete production in the ovaries can take place for at least four weeks. Thus, one of the more obvious explanations for sexual differences in Malpighian tubules is that the redirection of metabolism associated with egg production requires more elaborate excretory organs.

Another possibility is that the Malpighian tubules in the female are involved in a secondary function such as the storage of energy-rich substances. The primary storage organ is the fat body, but this decreases in size during the time of egg production. Thus the tubules may serve to store additional reserves to be utilized during the non-feeding late-nymph and adult stages.

It seems likely that the observations recorded here have some general significance, especially in Plecoptera. Sexual dimorphism in Malpighian tubules also occurs in *Pteronarcella badia* (Pteronarcidae), which differs in many life historical respects from *P. californica*. Further observations on other species would probably be rewarding.

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